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PENSOFT

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Return of the walking dead: First verified record of the shrew *Crocidura leucodon* (Hermann, 1780) in Hamburg, Germany

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http://zoobank.org/C34E0741-F7ED-482A-A014-082A464E571F

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Abstract

The bicolored shrew *Crocidura leucodon* so far has not been reported in Hamburg with certainty. Some plausible historical records before 1920 are present; in turn, two more recent records are doubtful for different reasons. Hence, the Red List status of the species for Hamburg has to be considered uncertain (either not present, or extinct). A citizen scientist provided a specimen of an unknown shrew, an accidental catch by a snap trap, to the Centrum für Naturkunde. The specimen was caught on the 12th of September 2019 in Tatenberg, Hamburg. It was morphologically and genetically clearly determined as *C. leucodon*. This find represents the first confirmed record of *C. leucodon* for the federal state of Hamburg and increases the number of shrew species recorded in Hamburg to five. The Red List status of the species will have to be reevaluated.

Key Words

Citizen Science, local extinction, Red List, rediscovery

Introduction

In Germany three genera of shrews can be found: *Crocidura, Sorex,* and *Neomys.* Within the genus *Crocidura* three species occur in Germany: *Crocidura suaveolens* (Pallas 1811), *C. leucodon* (Hermann 1780) and *C. russula* (Hermann 1780). While *C. russula* remains relatively common, the other two species are considered threatened, as they are adapted to agriculturally extensively used habitats. Although no native German shrew is a relevant species for the European Commission Habitats Directive (92/43/EEC), all shrew species in Germany are legally protected by the Bundesartenschutzverordnung (BArtSchV); but in the current Red List for the country four out of the five shrew species are considered least-concern species (Meinig et al. 2020). *Crocidura leucodon*'s Red List status in Germany was NT in 2020 (Near Threatened, German Red List "V").

Generally, all shrew species are more common in the southern and eastern parts of Germany with their density declining to the north. While *Crocidura suaveolens* is not found in northern Germany at all, the distribution of *C. leucodon* reaches the federal state Schleswig-Holstein. However, especially in the northern parts of the distribution, it occurs only scattered. In Schleswig-Holstein, the species is listed as vulnerable (Borkenhagen 2014); similarly, the species was listed as vulnerable in Lower Saxony and Bremen in 1991 (Heckenroth 1993; Meinig et al. 2020); in Hamburg, the species was even considered extinct (Fig. 1, Schäfers et al. 2016). However, so far no secure records of the bicolored shrew are available for Hamburg (Ebersbach 2015; Schäfers et al. 2016), suggesting the species may have never been present. Here, we report the first confirmed record of *C. leucodon* for Hamburg.

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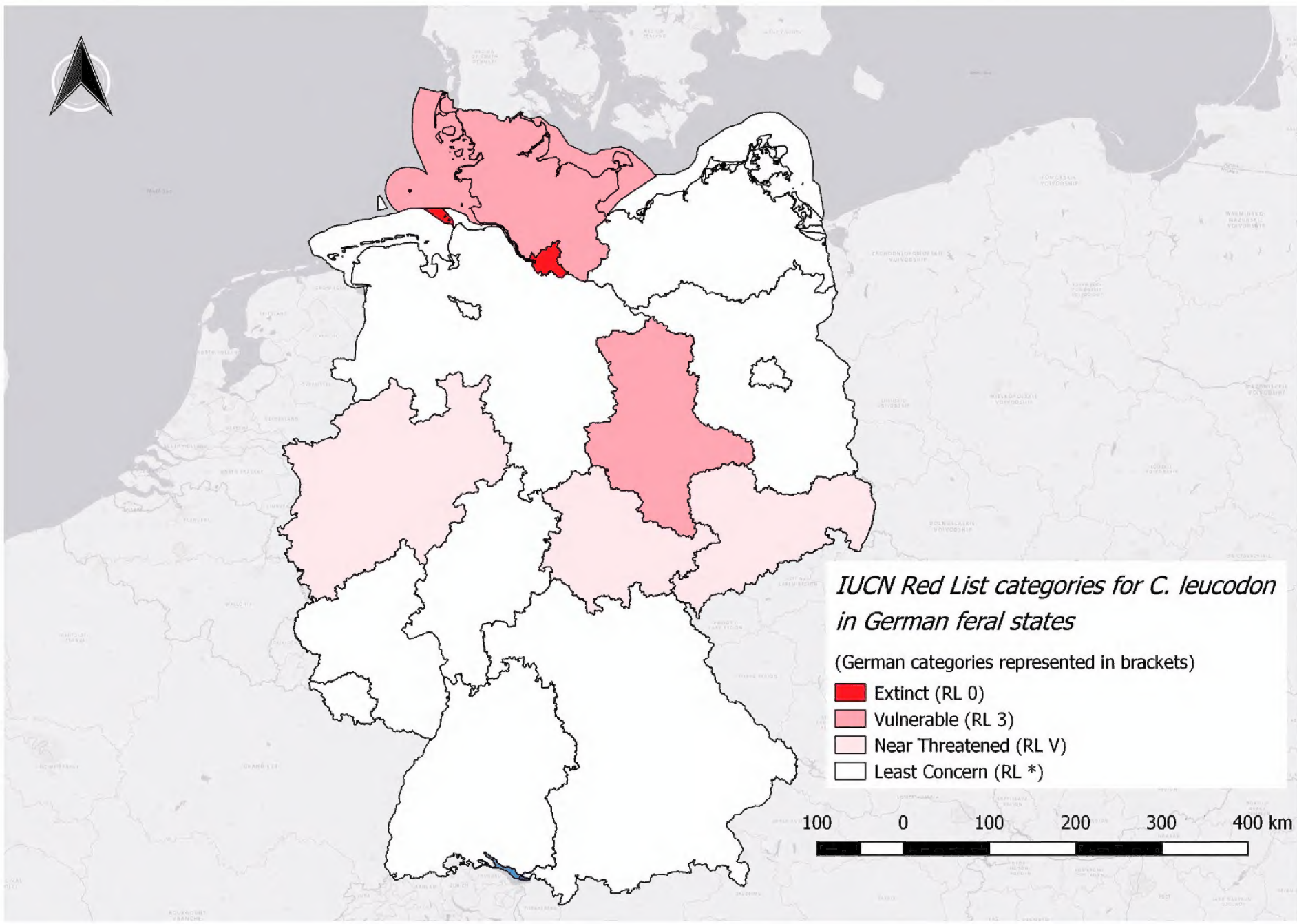
IUCN Red List categories for C. leucodon in German feral states
(German categories represented in brackets)
Extinct (RL 0)
Vulnerable (RL 3)
Near Threatened (RL V)
Least Concern (RL *)
100 0 100 200 300 400 km

Figure 1. Map of Red List categories of *C. leucodon* in the German federal states based on Meinig et al. (2020). The city federal state Bremen is included in Lower Saxony and has no separate Red List. All Red List categories were taken from the German Red List of mammals (Meinig et al. 2020), which includes an overview of all federal states.

Material and methods

Two potential specimens of *Crocidura leucodon* were donated to the Zoological Museum Hamburg. Specimen ZMH-T-3022 was caught in a snap trap on 09.12.2019 in a private barn in south eastern Hamburg (53°30'00.0"N, 10°04'48.0"E; see Fig. 2). The juvenile individual ZMH-T-3023 was found gasping on 12.07.2016 on a northern Hamburg private garden plot (53°34'48.0"N, 9°48'36.0"E), died shortly after and was subsequently donated to the CeNak collection. Both specimens were conserved in 70% ethanol.

Morphological identification was performed following the keys and descriptions implemented in Krapp (1990), Angermann (2012) and Schaefer (2009). In order to provide images of skeletal characters relevant for identification, micro-computed tomography (µCT) scans of the skull and thorax were performed with an YXLON FF20 CT (Yxlon International GmbH, Hamburg). We used the micro-focus setting with the following parameters: 80 kV, 40 mA, no filter, pixel size of 45.4 µm. Images were reconstructed using CERA (Siemens Healthcare GmbH, Erlangen) and processed using VGSTUDIO MAX (Volume Graphics, Heidelberg).

To verify the morphological diagnosis, we performed DNA barcoding. Total genomic DNA was extracted of a piece of tongue using a standard CTAB protocol (Borges et al. 2009). A fragment of the barcoding gene Cytochrome Oxydase I (COI) was amplified using the primers LCOJJ and HCOJJ (Astrin and Stüben 2008). PCR was performed with the following setup: 5.7 µl PCR grade water, 2 µl 5x buffer, 0.5 µl of each primer, 0.2 µl dNTP´s, 0.1 µl DreamTaq polymerase and 1 µl template. PCR conditions were as follows: activation step at 95 °C for 5 min, followed by 35 cycles of 30 sec. denaturation at 95 °C, 30 sec. annealing at 48 and 1 min elongation at 72 °C. Cycling was terminated by a final extension step at 72 °C for 10 min. PCR success was checked by gel electrophoresis and successful products were purified with an enzyme mix consisting of Exonuclease I and Shrimp-Alkaline Phosphatase (ExoSap). Amplicons were sent to Macrogen (Amsterdam, Netherlands) for sequencing.

The resulting chromatogram was checked, trimmed and proofread in Geneious v. 9 (Kearse et al. 2012). In a first step, we searched the cleaned sequence in NCBI BLAST (Geer et al. 2009), GBOL (Geiger et al. 2016) and BOLD (Ratnasingham and Hebert 2007) to confirm the determination of the specimen as *Crocidura leucodon*. Secondly, we reconstructed a phylogenetic tree including the different species of the genus native to Germany to see the phylogenetic position of our specimen to confirm the morphological identification. Several sequences of *Sorex minutus*,

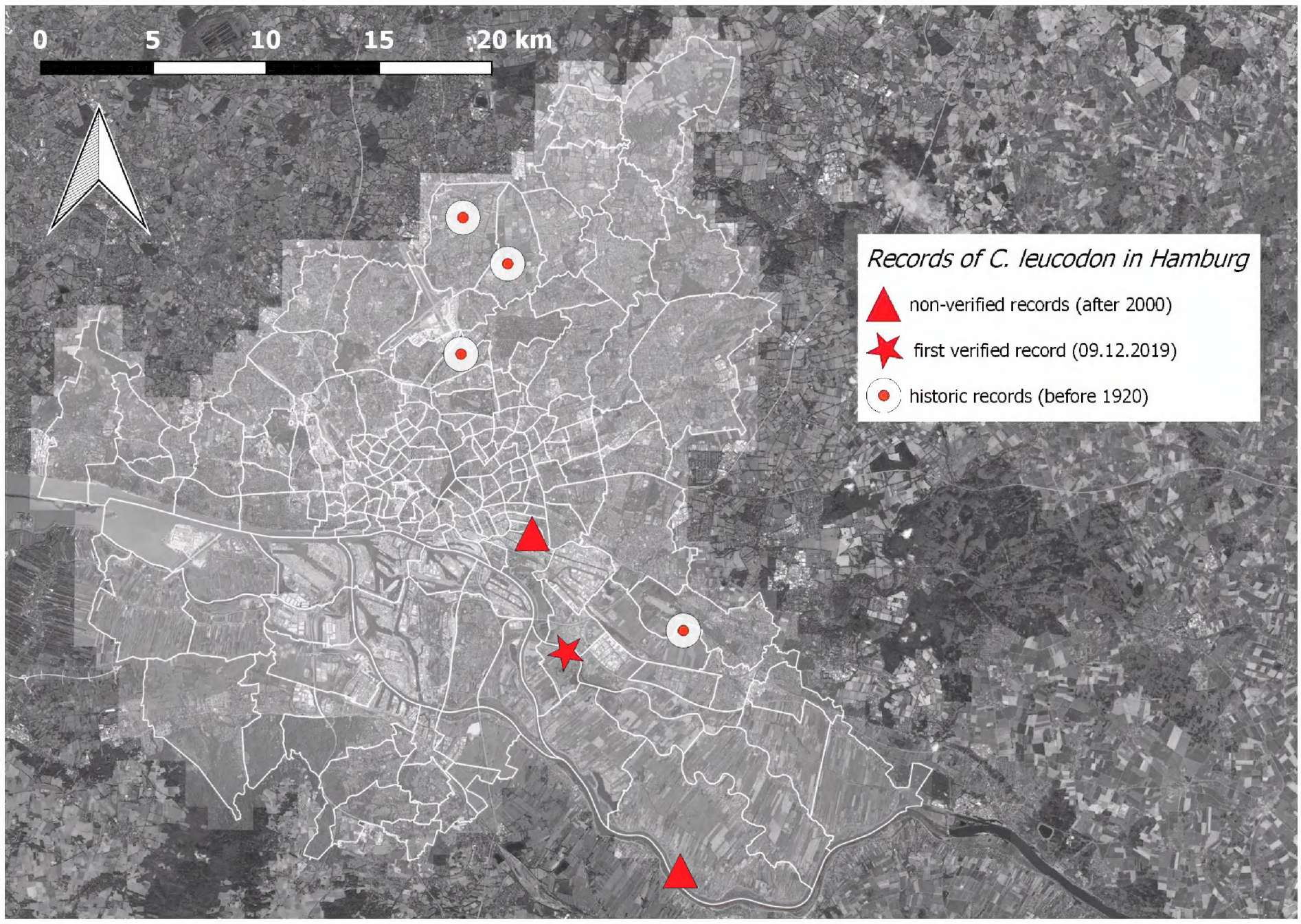
0 5 10 15 20 km
Records of C. leucodon in Hamburg
non-verified records (after 2000)
first verified record (09.12.2019)
historic records (before 1920)

Figure 2. Records of *C. leucodon* in Hamburg. Historic records (red dot) before 1920 are surrounded by a buffer zone as location data is not precise. Not verified records (red triangle) are doubtful, because of the lack of vouchers and unsecure identification. The only verified recent record (red star) is discussed in this paper.

Sorex araneus, *Neomys anomalus*, *Neomys fodiens*, *Crocidura suaveolens*, *Crocidura russula* and *C. leucodon* from both databases were downloaded and added to the dataset (Appendix 1: Table S1). MUSCLE (Edgar 2004), as implemented in Geneious was used to align all sequences. The resulting alignment was trimmed to similar length at 541 bp. The best substitution model was determined using the R package PHANGORN (Schliep et al. 2017) in mRAN v. 3.4 (Microsoft 2017) with RStudio v. 1.0.143 (Studio 2012). GTR was determined as best model. We generated a phylogenetic tree using RAxML-HPC2 (Stamatakis 2014) on XSEDE via Cipres Science Gateway (https://www.phylo.org/www.phylo.org).

All potential records of the bicolored shrew from Hamburg were plotted on a map (Fig. 2). Besides of the new record reported here, we included all historic and recent location data for *Crocidura leucodon* provided by the lower nature conservation authority in Hamburg (BUKEA). All plane-table sheet (TK25) locations were translated into coordinates by using the midpoints of the plane squares as GPS points. All coordinates were plotted on a map using QGis 2.18 (QGIS Development Team 2016); an ESRI satellite map (https://server.arcgisonline.com/ArcGIS/rest/services/World_Imagery/MapServer/tile/%7Bz%7D/%7By%7D/%7Bx%7D&zmax=20&zmin=0) map from QGis QickMapsServices 0.9.11.1 served as base map together with a layer of the city (free licence, Freie und Hansestadt Hamburg, Landesbetrieb Geoinformation und Vermessung 2016).

The current Red List categories for all federal states were derived from the German national Red List for mammals (Meinig et al. 2020) and plotted on a second map using an ESRI Gray light layer (http://services.arcgisonline.com/ArcGIS/rest/services/Canvas/World_Light_Gray_Base/MapServer/tile/{z}/{y}/{x}) and VG250_LAN layer for federal states from open governmental database (http://www.bkg.bund.de, GeoBasis-DE / BKG 2021).

Results

Morphological identification

All teeth of ZMH-T-3022 are of white color (Fig. 3) leading to the genus *Crocidura* in Germany. The sharp delimitation of dorsal and ventral fur coloration, which is a character of *Crocidura leucodon*, is clearly visible in dry condition of the voucher (in ethanol this is less clearly visible). In addition, the body size of 6.5 cm, the tail length of 3.2 cm and the hind foot length of 10.27 mm support the determination. This leads to a body length / tail length ratio of about 1:2, which is typical for *C. leucodon* (Krapp, 1990). The

Figure 3. Macroscopic focus stacked images of the *C. leucodon* voucher ZMH-T-3022 from Hamburg. The genus is identified by the clearly visible white teeth (**a, c**), and the presence of long hair on the tail (**b**). The tail is bicolored with a much darker upper side. The distance between urogenital opening and excretory opening is small (female) (**d**); whole specimen in lateral view, the tail length is almost half of the body length (**e**).

micro-CT-scan revealed a bland malar bone extension of the strongly reduced zygomatic arch. The ratio of the upper edge of the maxillary alveoles and the skull height above the canines (red square) is about 1:1. This is a specific trait of *C. leucodon* (Fig 4).

For ZMH-T-3023 the general habitus supports the determination of the specimen as *Crocidura russula*. The dorsal and ventral fur coloration delimitation was less sharp and the tail length to body length ratio was about 1:3. For the reason of uncertainties in morphology-based

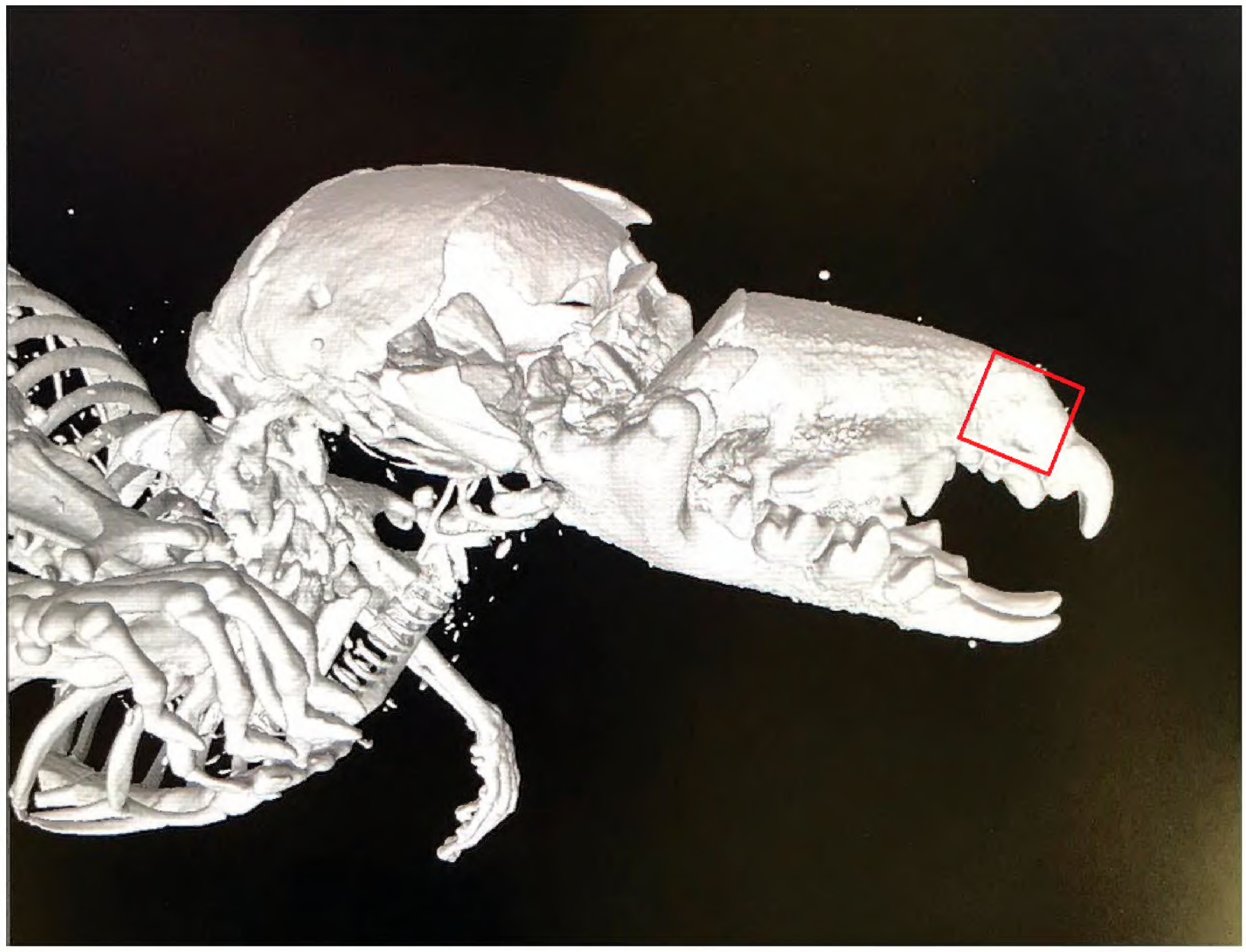

Figure 4. Micro-CT scan of the head of the specimen; the ratio of the upper edge of the alveoles in the maxilla and the skull height above the canines (red square) is about 1:1. The crack in the middle of the skull is a result of the snap trap.

determination and because the specimen is a juvenile, we rely on DNA barcoding for determination.

The DNA barcode of ZMH-T-3022 showed 99.67 - 100% identical bases for *Crocidura leucodon* in the top five hits in BOLD (Ratnasingham & Hebert 2007) and GBOL Geiger et al. 2016). The DNA barcode of ZMH-T-3023 showed 99.63–100% concurrence for *C. russula* in the top five hits in both databases. The specimens each joined highly supported clades (bootstrap value > 100%) of *C. leucodon* and *C. russula* sequences, respectively, in a phylogenetic tree of native shrew species based on COI (Fig. 5).

Discussion

Citizens of Hamburg provided two shrew specimens of unknown species assignment to the CeNak. One specimen was identified as *Crocidura leucodon*, the second as *C. russula* using a combination of morphological and genetic methods. While the latter is a species recently recorded (Schäfers et al. 2016), *C. leucodon* was considered locally "extinct" (RL category) in Hamburg, because no study undoubtedly reported the species in the state for one century.

The species was considered locally extinct (Red List 0), rather than absent in Hamburg, because of six past records for the state (Fig. 1): four before 1920, one in 2011 (Schäfers et al. 2016) and one in 2018 (database of the lower nature conservation authority of Hamburg, BUKEA Artenkataster). However, for none of these records, proper documentation exists and thus the finds cannot be confirmed and have to be regarded as doubtful.

In Hamburg, the white-toothed shrew *Crocidura russula* is native, but can potentially be confused with *C. leucodon*, because of a superficially similar morphology, especially when identified as living individuals (specifically juveniles) from a distance. Three of four historical records are from northern Hamburg (Itzerodt 1904, cited in Schäfers et al. 2016), where also *C. russula* is recorded (Schäfers et al. 2016). Only the record from eastern Hamburg before 1920 seems plausible in terms of the local habitats. This area has a lower human population density and is characterized by rather extensive agricultural land use representing typical *C. leucodon* habitat. Further, there are two additional recent records, which appear doubtful. In 2011, a pellet from a bird of prey or an owl was found in southeastern Hamburg (53.41, 10.18) near the Elbe, which is equivalent to the border of the federal state. The study of bones from the pellet identified *C. leucodon*. While in this case the identification is plausible, the original location of the specimen remains obscure. The recovery point of the pellet is less than 500 m away from the boarder to Lower Saxony. In this region of Lower Saxony, large scale agricultural land use provides typical habitat properties commonly associated with the demands of *C. leucodon*. The Elbe River represents a faunal barrier to terrestrial mammals, not so, however, to a bird

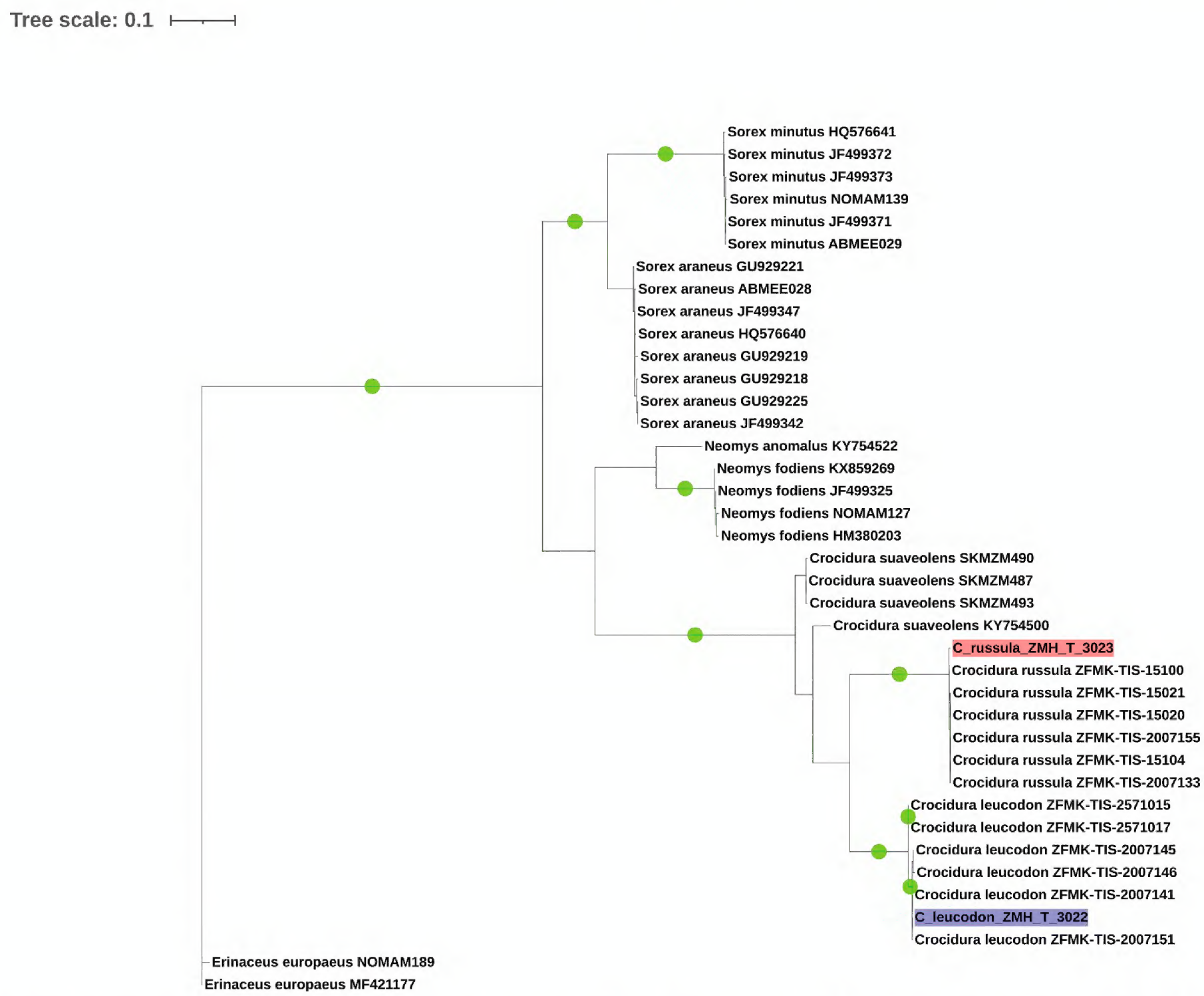

Figure 5. Phylogenetic tree (generated with RaxML) based on partial COI sequences including all native shrew species (excluding *Sorex alpinus*) from NCBI and BOLD (see Supplemental Table S1) and two sequences of white-toothed shrews (genus *Crocidura*) from Hamburg. ZMH-T-3023 clearly clusters in a clade with other *C. russula* sequences. ZMH-T-3022 falls within a clade of *C. leucodon* specimens. Green dots indicate bootstrap values > 95 %

of prey. A bird may thus have carried its prey from Lower Saxony into Hamburg, and hence, the record cannot securely be attributed to any federal state. In 2018, someone reported the sighting of seven individuals of *C. leucodon* at Billerhuder Island, a residential area surrounded by waterways. The settlement structure consists of houses with gardens, which potentially could provide a winter habitat. However, this island is far away from any extensively used areas covered with hedges and shrubbery (the typical habitat of *C. leucodon*) and hence it is difficult to explain, how a population of *C. leucodon* may have established here. Future live trapping needs to be performed to potentially confirm this record.

None of those former records have any importance for the Red List status of *Crocidura leucodon* in Hamburg, because of missing vouchers or other proof. Hence, we here provide the first verified and vouchered record for the federal state of Hamburg. Indeed, it generally is difficult to evaluate the significance of a Red List of a city state such as Hamburg, which is surrounded by two federal states with much larger area and more diverse habitats, i.e. Lower Saxony and Schleswig-Holstein. Nevertheless, our finding represents an important record of a small and relatively rare mammal in the vicinity of a metropole region and hence may contribute to the understanding of urban wildlife. Further, our study will aid to better evaluate the Red List status for the bicolored shrew in Hamburg; the status as extinct will have to be revoked and the species has to be re-evaluated and may have to be considered threatened by extinction. Finally, our study shows, once again, the importance of interested citizen scientists for our understanding of the distributions of species, (not only) in the urban context.

Acknowledgement

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Appendix 1

Table S1. List of sequences from derived from GBOL, NCBI and BOLD databases providing the name of the taxon, the sequence ID and the source of the sequence.

Name of species	Sequence_ID	Source	Trimmed length
Crocidura leucodon	ZFMK-TIS-2007141	GBOL(German Barcode of Life)	541
	ZFMK-TIS-2007145	GBOL(German Barcode of Life)	541
	ZFMK-TIS-2007146	GBOL(German Barcode of Life)	541
	ZFMK-TIS-2007151	GBOL(German Barcode of Life)	541
	ZFMK-TIS-2571015	GBOL(German Barcode of Life)	541
	ZFMK-TIS-2571017	GBOL(German Barcode of Life)	541
Crocidura russula	ZFMK-TIS-15020	GBOL(German Barcode of Life)	541
	ZFMK-TIS-15021	GBOL(German Barcode of Life)	541
	ZFMK-TIS-15100	GBOL(German Barcode of Life)	541
	ZFMK-TIS-15104	GBOL(German Barcode of Life)	541
	ZFMK-TIS-2007133	GBOL(German Barcode of Life)	541
	ZFMK-TIS-2007155	GBOL(German Barcode of Life)	541
Crocidura suaveolens	KY754500	NCBI(GenBank)	541
	BOLD: SKMZM487	BOLD (Barcode of Life)	541
	BOLD: SKMZM488	BOLD (Barcode of Life)	541
	BOLD: SKMZM489	BOLD (Barcode of Life)	541
Erinaceus europaeus	MF421177	NCBI(GenBank)	541
	NOMAM189	NCBI(GenBank)	541
Neomys anomalus	KY754522	NCBI(GenBank)	541
Neomys fodiens	HM380203	NCBI(GenBank)	541
	JF499325	NCBI(GenBank)	541
	KX859269	NCBI(GenBank)	541
	NOMAM127	NCBI(GenBank)	541
Sorex araneus	BOLD: ABMEE028	BOLD (Barcode of Life)	541
	BOLD: GU929218	BOLD (Barcode of Life)	541
	BOLD: GU929219	BOLD (Barcode of Life)	541
	BOLD: GU929221	BOLD (Barcode of Life)	541
	BOLD: GU929225	BOLD (Barcode of Life)	541
	HQ576640	NCBI(GenBank)	541
	JF499342	NCBI(GenBank)	541
	JF499347	NCBI(GenBank)	541
Sorex minutus	BOLD: ABMEE029	BOLD (Barcode of Life)	541
	HQ576641	NCBI(GenBank)	541
	JF499371	NCBI(GenBank)	541
	JF499372	NCBI(GenBank)	541
	JF499373	NCBI(GenBank)	541
	NOMAM139	NCBI(GenBank)	541